

Effect of Host Genotypes and Weather Variables on the Severity and Temporal Dynamics of Sorghum Anthracnose in Ethiopia

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Abstract: The severity and temporal dynamics of anthracnose on susceptible (BTx623 and AL70) and resistant lines (2001 PWColl No. 022 and 2001 HarargheColl No. 12) were studied in field plots during the 2007 and 2008 growing seasons in southern Ethiopia. The initial, final and mean anthracnose severities and area under disease progress curves were used as criteria to evaluate the response of the genotypes. Over the two years, the initial, final and mean anthracnose severities ranged from 0.88 to 16.13%, 7.56 to 78.38% and 3.57 to 46%, respectively, while area under disease progress curve averaged for the two years ranged from 221.31 to 2951.88. All the evaluation criteria showed highly significant variations ($p < 0.0001$) among the genotypes and the Ethiopian genotype 2001 PWColl No. 022 consistently had the lowest disease levels regardless of the evaluation criteria and growing season. The disease appeared rather late and progressed slowly on this genotype. On the other hand, the exotic cultivar, BTx623, showed the most severe anthracnose infection. Initial anthracnose severity was significantly higher and the disease developed rapidly on BTx623 than on the other three genotypes. The other two genotypes showed intermediate response and progression of the disease. Correlation and regression analyses revealed a significantly strong association between rainfall and anthracnose severity but temperature appeared to have little/no impact on the development of anthracnose in the field. The present study confirmed the effect of both host genotypes and weather, particularly rain on anthracnose development. The Ethiopian sorghum genotype 2001 PWColl No. 022 was recommended as stable source of resistance against this important disease.

Key words: Area under disease progress curve, *C. sublineolum*, environment, resistance, susceptible

INTRODUCTION

Sorghum is a major staple food crop for millions of people especially in the developing world (FAO, 2009). Ethiopia ranks eighth in total production of the crop, which amounts to 2.8 million tons per annum on about 1.6 million ha of cultivated land (FAO, 2009; CSA, 2009). This makes sorghum the fourth most important crop next to maize (*Zea mays*), teff (*Eragrostis tef*) and wheat (*Triticum* species) in terms of both area planted and total production (CSA, 2009). Sorghum in Ethiopia is mainly grown by subsistence farmers either as a sole crop or intercropped with other field crops including maize, teff and beans and to some extent with chat (*Chata edulis*). Due to the diverse nature of the farming systems and

climatic conditions under which sorghum is grown, the production of sorghum in Ethiopia is adversely affected by several biotic and abiotic constraints among which the disease anthracnose is the major one (Hulluka and Esele, 1992; Chala *et al.*, 2007). Sorghum anthracnose is caused by a fungal pathogen *Colletotrichum sublineolum* and since its report in 1960 (Sutton, 1980), the disease has been identified as one of the major factors constraining sorghum production worldwide (Pande *et al.*, 1993; Thomas *et al.*, 1996; Thakur and Mathur, 2000). On infected sorghum in the field, anthracnose can cause yield losses of 50% or more (Harris *et al.*, 1964; Ferreira and Warren, 1982). The development of anthracnose in sorghum fields largely depends on host susceptibility and prevailing weather conditions (Pastor-Corrales and

Frederiksen, 1980; Ali and Warren, 1987; Néya and Normand, 1998; Marley *et al.*, 2001; Hess *et al.*, 2002; Erpelding and Prom, 2004, 2006; Erpelding and Wang, 2007). Cultural practices including residue and weed management, planting disease free seeds, crop rotation are also known to impact anthracnose development in the field (Warren, 1986; Cardwell *et al.*, 1989; Casela and Frederiksen, 1993; Somda *et al.*, 2007).

Currently the management of sorghum anthracnose largely depends on the deployment of resistant varieties. In addition, the choice of planting dates is also found to significantly impact the development of the disease (Ngugi *et al.*, 2000; Marley, 2004) and hence could play a role in reducing anthracnose severity. Furthermore, understanding the temporal dynamics of the disease is an essential task to identify the proper planting time, which in turn contributes towards developing effective, affordable, safe and sustainable management strategies. Studies on the temporal progress of plant diseases have yielded substantial knowledge on their epidemiology, which is essential to design proper management practices (Olatinwo *et al.*, 1999; Benson *et al.*, 2006; Mouen-Badimo *et al.*, 2007). Thus, the current project was designed to study the severity and temporal dynamics of anthracnose on different sorghum genotypes and additionally, to determine the impact of weather variables on anthracnose development.

MATERIALS AND METHODS

Experimental site: The experiment was conducted in 2007 and 2008 sorghum growing seasons in Southern Ethiopia. The area is characterized by high rainfall (>1200 mm annum⁻¹) and moderate temperature (18.5°C) based on 11 year data. The research sited is located between 6°59.098' N latitude and 37°52.645' E longitude and has an altitude of 1947 m.a.s.l. The area is known to be suitable for anthracnose development based on existing weather conditions and previous study (Chala *et al.*, 2007).

Planting, experimental design and inoculation: Three sorghum genotypes of Ethiopian origin and a universally susceptible cultivar, BTx623, were evaluated for their reaction to anthracnose in 2007 and 2008 cropping seasons. Planting was conducted in April and May of 2007 and 2008, respectively, in plots of 2.5×3 m at spacing of 75×15 cm. The experiment was laid out in randomized complete block design with four replications. The 2008 planting time was delayed due to late onset of rainfall. All the management practices including hand weeding were carried out as needed and an insecticide, Endosulfan 35%

EC, was applied at a rate of 21 t ha⁻¹ at 4 and 6 weeks after germination to control of stalk borers. Disease assessments were conducted on the naturally-infected plants in both years.

Data collection and analysis: Monthly weather data of 11 consecutive years (1997-2008) of the area were obtained from the National Meteorological Agency.

Data were collected on anthracnose severity as percentage of leaf area covered by the symptom at 10 days interval for eight and seven consecutive times in 2007 and 2008, respectively, starting from the onset of clear symptoms on at least two of the sorghum genotypes. All the plants within the inner two rows of each plot were used for data collection. Area Under Disease Progress Curve (AUDPC) was calculated from the severity data following the method proposed by Madden *et al.* (2008) with some modifications as:

$$AUPDC = \sum_{i=1}^{n-1} [(X_i + X_{i+1}) / 2] (t_{i+1} - t_i)$$

Where:

X = Disease severity

t_i = Time in days of the ith assessment from the first assessment date

n = Total number of assessments.

Initial Anthracnose Severity (IAS) (the first severity record at 60 days post planting), Final Anthracnose Severity (FAS), Mean Anthracnose Severity (MAS) and AUDPC were analyzed statistically using SAS computer package, version 9.11 (SAS Institute Inc, 2003). LSD test at the 0.05 probability level was used for mean comparisons. Correlation analysis was performed using PROC CORR procedure of the SAS computer package to determine the relationship among the different disease parameters and weather variables. A similar regression analysis procedure used by Tarekegn *et al.* (2006) was applied to further elucidate the effect of rainfall and temperature on the severity of anthracnose. The regression analysis was performed between rainfall and temperature conditions of each month and average anthracnose severity record of the same month.

RESULTS

Weather conditions: The research area generally received a high but variable rainfall during the 2007 and 2008 experimental seasons. Overall total rainfall was higher during the 2007 experimental season than in 2008 and the 11 year average rainfall of the area. The total monthly

Table 1: Weather parameters of the research site during the 2007 and 2008 growing seasons

Months	Rainfall (mm)			Temperature (°C)								
				Maximum			Minimum			Average		
	2007	2008	11 year	2007	2008	11 year	2007	2008	11 year	2007	2008	11 year
April	158.10	74.10	174.1	25.2	26.7	25.7	14.4	14.4	14.3	19.8	20.6	20.0
May	213.70	108.8	169.4	25.1	24.2	24.6	14.5	13.9	14.2	19.8	19.0	19.4
June	198.80	100.1	117.1	22.1	22.4	22.6	13.7	17.7	13.5	17.9	20.1	18.1
July	207.50	146.1	171.8	21.1	20.7	21.2	12.9	13.0	13.2	17.0	16.8	17.2
August	229.00	183.9	152.3	21.1	21.4	21.7	13.6	13.3	13.2	17.4	17.3	17.5
September	269.30	129.7	116.8	22.6	23.3	23.4	13.7	14.0	13.4	18.1	18.6	18.4
October	21.100	159.9	112.9	24.7	24.4	24.4	12.8	13.1	13.2	18.8	18.8	18.8
Total	1297.5	902.6	1262	NA	-	-	-	-	-	-	-	-
Mean	185.40	128.9	144.9	23.1	23.3	23.4	13.6	14.2	13.6	18.4	18.7	18.5

NA: Not applicable

Table 2: Combined analysis of variance for Initial Anthracnose Severity (IAS), Final Anthracnose Severity (FAS), Mean Anthracnose Severity (MAS) and Area Under Disease Progress Curve (AUDPC) of four sorghum lines tested in 2007 and 2008 cropping seasons

Parameters	Source	df	Sum square	Mean Squar	F-value	Pr>F
IAS	Year	1	227.111328	227.111328	81.37	<0.0001
	Genotype	3	1162.474609	387.491536	138.84	<0.0001
	Year*Genotype	3	417.771484	139.257161	49.89	<0.0001
	Rep (Year)	6	11.183594	1.863932	0.67	0.6767
FAS	Year	1	123.95251	123.95251	14.52	0.0013
	Genotype	3	23587.70254	7862.56751	920.87	<0.0001
	Year*Genotype	3	50.88754	16.96251	1.99	0.1521
	Rep (Year)	6	39.13896	6.52316	0.76	0.6075
MAS	Year	1	185.710531	185.710531	51.45	<0.0001
	Genotype	3	8304.817761	2768.272587	766.89	<0.0001
	Year*Genotype	3	91.691616	30.563872	8.47	0.0010
	Rep (Year)	6	8.396505	1.399418	0.39	0.8773
AUDPC	Year	1	79036.96	79036.96	4.74	0.0430
	Genotype	3	34533033.74	11511011.25	690.56	<0.0001
	Year*Genotype	3	40250.51	13416.84	0.80	0.5074
	Rep (Year)	6	41013.73	6835.62	0.41	0.8627

Table 3: Initial Anthracnose Severity (IAS) at 60 days post planting, Final Anthracnose Severity (FAS) at 130 days after planting, Mean Anthracnose Severity (MAS) and Area Under Disease Progress Curve (AUDPC) of sorghum genotypes

Genotypes	IAS (%)			FAS (%)			MAS (%)			AUDPC		
	2007	2008	Mean	2007	2008	Mean	2007	2008	Mean	2007	2008	Mean
BTx623	7.25a ¹	25.00a	16.13a	78.00a	78.75a	78.38a	40.84a	51.16a	46.00a	2841.25a	3062.5a	2951.88a
AL70	3.00b	4.06b	3.53b	53.25b	60.75b	57.00b	22.88b	27.68b	25.28b	1548.75b	1613.6b	1581.19b
2001 HarargheColl No. 12	1.50c	4.00b	2.75b	24.88c	29.75c	27.32c	10.11c	12.71c	11.41c	676.85c	720.9c	698.89c
2001 PWColl No. 022	0.88c	0.88b	0.88c	6.25d	8.88d	7.56d	2.79d	4.34d	3.57d	187.63d	255.0d	221.31d
Average	3.15	8.48	5.82	40.6	44.53	42.56	19.16	23.97	21.56	1313.62	1413.02	1363.317
p-value	<0.0001	<0.0001		<0.0001	<0.0001		<0.0001	<0.0001		<0.0001	<0.0001	

¹Means within a column followed by the same letter(s) are not significantly different at the 5% probability level based on LSD for multiple comparisons

rainfall of the research area varied from 21 to 269 mm in 2007 and from 74 to 184 mm in 2008 growing seasons, while the 11 year monthly rainfall ranged from 112 to 174 mm (Table 1). Total rainfall was lower in most months of 2008 as compared to that of 2007 and the 11 year average. However, minimum, maximum and average temperature of the area showed little or no variability during all the experimental months except in June 2008, when the maximum temperature was much higher than that of 2007 and the 11 year average. Overall, the area was found to have a favorable climate for the development of sorghum anthracnose.

Disease assessment: None of the tested sorghum genotypes was immune from anthracnose infection in any

of the experimental seasons. However, IAS and MAS were significantly affected by genotype ($p < 0.01$) and genotype by year interaction ($p < 0.01$) (Table 2). The final anthracnose severity also was significantly affected by genotype ($p < 0.01$). Significant genotype by year interaction may indicate that the genotypes responded differently during the 2007 and 2008 growing seasons.

Disease severity: The disease severity was based on a scale of 1-100%. IAS, FAS and MAS averaged 3, 41 and 19% in 2007 and 9, 45 and 24% in 2008, respectively (Table 3), suggesting the presence of enough disease pressure in the research area that enabled for successful evaluation in both years. In 2007 experimental season, IAS, FAS and MAS ranged from 0.9 to 7.3%, 6.3 to 78%

and 2.8 to 40.8%, respectively and showed highly significant ($p < 0.0001$) variations among the tested genotypes. These three severity records were all lowest on 2001 PWColl No. 022 followed by 2001 HararghieColl No. 12 and AL70, while the exotic cultivar, BTx623, had the highest severity records at any time of assessment.

Overall disease pressure was higher in 2008 than in 2007 with IAS, FAS and MAS varying from 0.9 to 25%, 8.9 to 78.8% and 4.3 to 51.2%, respectively. This indicated a 35-245, 0.96-14.1 and 25.7-55.6% increase in IAS, FAS and MAS, respectively. The genotypes differed significantly ($p < 0.0001$) in all the severity records and 2001 PWColl No. 022 had the lowest IAS, FAS and MAS in 2008 followed by 2001 HararghieColl No. 12 and AL70. Again, BTx623 exhibited the highest IAS, FAS and MAS. Thus, confirming its susceptibility to anthracnose.

AUDPC: AUDPC was calculated for each genotype and found to be highly significant. The AUDPC values varied between 188 and 2841 in 2007 and from 255 to 3063 in 2008 experiment. The lowest AUDPC values in both years were obtained from the genotype 2001 PWColl No. 022 followed by 2001 HararghieColl No. 12 and AL70. The highest and most significant AUDPC values in both 2007 and 2008 experimental seasons were calculated for BTx623 and results of this analysis were in line with those obtained from the various severity records.

Overall, results of the two years experiments suggest a differential but stable reaction by the genotypes to natural infection by anthracnose.

Temporal dynamics: Both the onset and rate of progress of anthracnose varied among the tested genotypes in both experimental years. The disease started earlier (data not shown) and progressed very rapidly on BTx623, while it showed a relatively late appearance and slow development on the genotypes 2001PWColl#022 and 2001 HararghieColl No. 12. The local susceptible check, AL70, had a somewhat intermediate anthracnose development both in 2007 and 2008.

During the first assessment, the severity of anthracnose on BTx623 was around 7 and 25% in 2007 and 2008, respectively. Data collected at 10 days interval showed remarkable increase in anthracnose severity (4.5-16% in 2007 and 0.3-13.5% in 2008) on BTx623, with the highest increase in severity occurring during late developmental stages of the plants. This period coincided with the end of August and beginning of October in 2007 and 2008, respectively (Fig. 1). On the other hand, the resistant genotype 2001 PWColl No. 022 exhibited anthracnose severity of 0.9% at the first assessment in both years. The increase in anthracnose severity for this

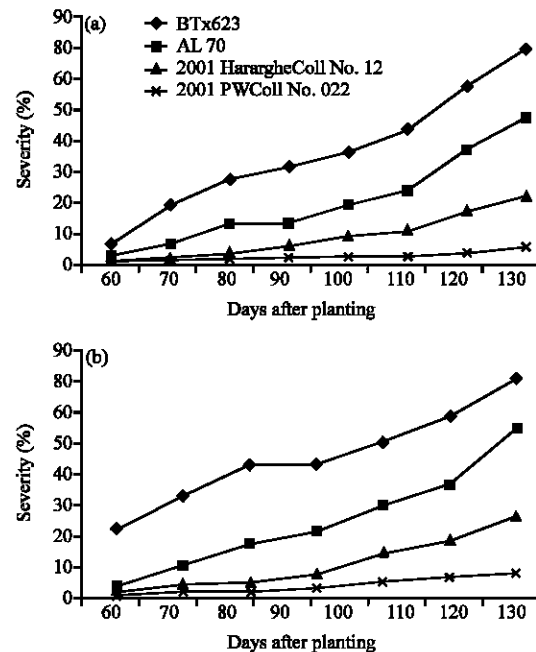


Fig. 1: Disease progress curves of anthracnose on four sorghum genotypes in (a) 2007 and (b) 2008 growing seasons

resistant line at 10 days interval was -0.2-2.3 and 0-2.4% in 2007 and 2008, respectively. The highest increase in anthracnose severity on 2001 PWColl No. 022 was recorded in September of each year. The remaining two genotypes i.e., AL70 and 2001 HararghieColl No. 12 showed an intermediate increase in anthracnose severity with the former being more susceptible and showing a more rapid disease increase than the later.

Results of this study generally suggest the time frame between August and end of September as the most important period for anthracnose development on sorghum.

Correlation and Regression analysis: Correlation coefficients between the various evaluation criteria based on the two years averaged data varied from 0.67 to 0.99, suggesting a highly significant ($p < 0.0001$) association between the parameters (Table 4). This may indicate the possibility of using any of the parameters as evaluation criteria but care must be taken with the IAS as it might be linked with age related resistance.

Correlation and regression analyses between mean anthracnose severity and weather variables were conducted only on the 2007 data due to the very variable weather condition in 2008, which makes this year's analysis much more difficult. Results of both analyses revealed a positive and significant relationship between

Table 4: Pearson's correlation coefficients between Initial Anthracnose Severity (IAS), Final Anthracnose Severity (FAS), Mean Anthracnose Severity (MAS) and Area Under Disease Progress curve (AUDPC) over the two years

Severities	FAS	MAS	AUDPC
IAS	0.67***	0.83***	0.78***
FAS	-	0.96***	0.97***
MAS	0.96***	-	0.99***

***Significant at $p < 0.0001$

Table 5: Pearson's correlation and regression analyses between mean anthracnose severity and weather parameters

Genotype				
Statistical analysis	BTx623	AL70	2001 HararghieColl No. 12	2001 PWCol No. 022
r				
TRF	0.96911*	0.99299**	0.99321**	0.99740**
MinT	0.21480 ^{NS}	0.33289 ^{NS}	0.39538 ^{NS}	0.30860 ^{NS}
MaxT	0.32171 ^{NS}	0.44553 ^{NS}	0.47703 ^{NS}	0.51855 ^{NS}
AveT	0.30941 ^{NS}	0.44154 ^{NS}	0.48710 ^{NS}	0.48487 ^{NS}
R²				
TRF	0.94	0.99	0.98	0.99
MinT	0.05	0.11	0.16	0.10
MaxT	0.10	0.20	0.23	0.27
AveT	0.10	0.20	0.24	0.24

r: Correlation coefficient; TRF: Total rainfall; MinT: Minimum temperature; MaxT: Maximum temperature; AveT: Average temperature; R²: Coefficient of determination of the regression analysis. *Significant at $p < 0.05$, **Significant at $p < 0.01$. NS: Not significant

rainfall and anthracnose severity but a rather weak and none significant relationship between temperature and disease severity (Table 5). This indicates that rainfall could be a better predictor of anthracnose development than temperature.

DISCUSSION

The bulk of sorghum especially for food purpose is produced by low income subsistence farmers in the developing world, who can't afford high input agriculture like the use of fungicides. Thus, there is a constant demand for efficient and yet less costly control measures against sorghum diseases, including anthracnose which has long been known to pose a major threat to sorghum production in many parts of the world (Hulluka and Esele, 1992; Casela *et al.*, 1993; Mukuru, 1993). Hence, research in identifying sources of anthracnose resistance has been and continues to be conducted in many countries. New sources of anthracnose resistant lines have been identified across the world (Casela *et al.*, 2001; Hess *et al.*, 2002; Erpelding and Prom, 2004, 2006; Mehta *et al.*, 2005). Research results, which are yet to be published, have also confirmed the presence of wide sources of resistance to anthracnose among Ethiopian sorghum accessions. Results of the current research also gave a good indication for the existence of varying levels of resistance among the three sorghum genotypes used in this

experiment as evidenced by the highly significant variations in terms of disease severity and AUDPC. The most resistant genotype (2001 PWColl No. 022) reduced anthracnose by several folds as compared to the susceptible check, BTx623, suggesting its potential as good source of resistance. This genotype was collected from Northwest Ethiopia, a region which has quite optimum environmental conditions for anthracnose and where sorghum has been under cultivation for several hundreds if not thousands of years. Sorghum genotypes in this region might have been co-evolved along with pathogens infecting sorghum and this could lead to the selection of resistant genotypes for current production. Host-pathogen co-evolution is one of the various mechanisms leading to development of resistance as suggested by previous findings including Dogget (1980). However, the use of resistant genotypes against anthracnose has its own limitations due to high variability and virulence patterns in the pathogen population (Cardwell *et al.*, 1989; Browning *et al.*, 1999; Latha *et al.*, 2003), which may cause breakage of resistance. In this study, the most resistant genotype 2001 PWColl No. 022, retained its resistance to anthracnose despite the fact that it was collected from southern Ethiopia (more than 800 km), a hotbed for anthracnose. This suggests that there may be additional resistant sources from the region. Further research may be needed to affirm this in the future.

Other research findings have reported sowing dates as having significant impact on the severity of plant diseases, including anthracnose (Ngugi *et al.*, 2000; Marley, 2004; Park *et al.*, 2005). Thus, change in planting dates can serve as an alternative means of managing anthracnose in farmers' fields. However, for planting dates to be used as a viable option in disease management, one has to first determine the cycle of the disease in question and find out the optimum time when the disease reaches its peak levels. Our current findings showed the differential impact of genotypes with varying levels of resistance on the timely development/progress of anthracnose in a conducive environment. While variations in disease progress were remarkable among the genotypes with anthracnose showing a very slow progress on the genotype 2001 PWColl No. 022, the duration between August and end of September was found to be a peak time for anthracnose development in the field. This could be very much related to the environmental conditions that prevail during this time in addition to the life cycle of the pathogen and developmental stage of the crop, which both have to be studied in detail in the future. Based on our present results, it's likely that early planting may enable sorghum

to escape the high and most damaging levels of anthracnose. But further research is needed to precisely identify the best planting time to lessen the impact of the disease.

Suppressing the development of anthracnose has been considered as one useful strategy in the management of this important disease. Gwary and Asala (2006) achieved this through the application of fungicides, while Casela *et al.* (1993) and Marley *et al.* (2001) reported a slowed development of anthracnose as useful mechanism of resistance in some cultivars. Present results confirm a remarkable arrest in the temporal progress of anthracnose by the genotype 2001 PWColl No. 022, which was another evidence for this genotype to be considered as a useful source of resistance against anthracnose.

Weather conditions including rainfall and temperature are also known to influence the development of plant diseases in different pathosystems (Estrada *et al.*, 2000; Tarekegn *et al.*, 2006; Harikrishnan and del Río, 2008). The prevailing conditions during the course of the research were very favorable to the initiation and further progress of anthracnose as evidenced by the higher disease pressure of up to 79% severity and rapidly increasing disease severities especially on the susceptible genotypes. Although rainfall was generally more variable and less intense in 2008 than in 2007 and the 11 year average in present research area, it actually exceeded those recorded and reported as optimal amounts in other parts of the world (Néya and Normand, 1998; Hess *et al.*, 2002).

There exist some contradictions as to which weather variables are most suitable to anthracnose. While Ali and Warren (1987) reported warm and humid weather as most favorable to sorghum anthracnose, Erpelding and Wang (2007) found out low temperature as more conducive to the disease. Correlation and regression analyses made on our data clearly showed a strong and positive association between anthracnose severity and total rainfall. However, present results revealed no significant impact of temperature on anthracnose development. Hence, we conclude that rainfall could be the most decisive weather variable affecting anthracnose development in sorghum fields. But the temperature range on which our analysis was based was rather narrow and hence values well below and above those recorded for our research area should be considered to reach a more conclusive result in the future.

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